510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k041090

B. Purpose for Submission:
New device

C. Measurand:
High density lipoprotein cholesterol

D. Type of Test:
Quantitative, LDL and VLDL Precipitation, Cholesterol via Esterase-Oxidase, HDL

E. Applicant:
Denka Seiken Co., Ltd.

F. Proprietary and Established Names:
HDL-EX SEIKEN Assay Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR 862.1475, Lipoprotein test system

2. Classification:
   I (reserved per 862.9(c)(4) and (5))

3. Product code:
   LBS

4. Panel:
   Chemistry (75)

H. Intended Use:

1. Intended use(s):
   The HDL-EX SEIKEN Assay Kit is an *in vitro* diagnostic test for the quantitative determination of high-density lipoprotein cholesterol (HDL-C) in human serum and heparinized- or EDTA-plasma on automated chemistry analyzers in clinical laboratories.

2. Indication(s) for use:
   The HDL-EX SEIKEN Assay Kit is an *in vitro* diagnostic test for the quantitative determination of high-density lipoprotein cholesterol (HDL-C) in human serum and heparinized- or EDTA-plasma. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus, atherosclerosis and various liver and renal diseases).

3. Special conditions for use statement(s):
   Prescription use

4. Special instrument requirements:
   Automated chemistry analyzers e.g., Hitachi (917, 717, and 911)

I. Device Description:

The kit consists of Reagent-1, Reagent-2, and Lipid Calibrator. Reagent-1 contains the following: Buffer, cholesterol esterase (yeast), cholesterol oxidase (bacteria), catalase (bovine liver), and the sodium salt of N-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS). Reagent-2 contains the following: Buffer, peroxidase (horseradish), 4-aminoantipyrine, sodium azide, and surfactants (polyoxyethylene). Reagent-1 and Reagent-2 are supplied ready to use. The Lipid Calibrator is a preparation of lyophilized human serum containing various lipoproteins including HDL. Each serum donor unit used was tested by FDA-approved methods and found negative for HBs antigen, HIV and HCV antibodies.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Ultra N-Geneous HDL Cholesterol Reagent

2. Predicate 510(k) number(s):
   k021316

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Determination of high-density lipoprotein cholesterol (HDL-C)	Same
Specimen types	Serum, heparinized plasma, and EDTA plasma	Same

Similarities		
Item	Device	Predicate
Methodology	Two step homogenous method utilizing a detergent (surfactant) to directly measure HDL-C in a mixture of various lipoproteins, via esterase-oxidase	Same
Endpoint	Color development	Same
Differences		
Item	Device	Predicate
Linearity	1-150 mg/dL	3.7-200 mg/dL

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

The assay consists of two steps and uses a surfactant that specifically decomposes HDL particles but not the other lipoproteins.

In the first step, non-HDL lipoproteins complexes (chylomicrons, VLDL, IDL, and LDL) are dissolved in the buffer in Reagent-1. The cholesterol released from such non-HDL lipoproteins is hydrolyzed by the cholesterol esterase and then oxidized to cholestenone by the cholesterol oxidase. The hydrogen peroxide produced in the oxidation is converted to water and oxygen by the catalase.

In the second step, the HDL-specific surfactant in Reagent-2, releases cholesterol only from HDL particles. The cholesterol released is then subject to the same enzymatic reactions described above, catalyze by cholesterol esterase and cholesterol oxidase. In this case the presence of sodium azide inhibits the catalase allowing the peroxidase to catalyze a reaction between hydrogen peroxide, 4-aminoantipyrine and a derivatized aniline to produce a chromogenic compound.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   All performance evaluations were performed on a Hitachi 917 automated analyzer.

   a. Precision/Reproducibility:

   Within-run precision was assessed using control fluid at three different HDL-C levels. Each level was measured twenty times. The results were as follows:

	Low	Medium	High
N	20	20	20
Mean	29.8	56.0	92.1
SD	0.24	0.38	0.39
CV	0.79%	0.67%	0.42%

Between-run precision was assessed using control fluid at three different HDL-C levels. Each level was measured using a single lot of the assay reagent in one run per day for consecutive 20 days. The measurement was carried out by a single determination in each run. The results were as follows:

	Low	Medium	High
N	20	20	20
Mean	29.0	55.3	88.8
SD	0.47	0.89	1.49
CV	1.62%	1.60%	1.68%

b. *Linearity/assay reportable range:*
Linearity was assessed using equally spaced serial dilutions of serum-based high and low controls spiked with purified human HDL. The serial dilutions of the control sera were prepared using saline as the diluent. Comparison of the observed concentrations with the theoretical concentrations showed bias within plus or minus 5% at least up to 150 mg/dL. The recoveries supporting linearity in the lower limit ranged between 96.9% and 100.5%. The recoveries supporting linearity over the entire assay ranged between 98.4% and 101.3%.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
HDL-C value is assigned to the Lipid Calibrator with multi-lots of the proposed device and with the Primary Serum Calibrator. The Primary Serum Calibrator is a preparation of well-characterized serum pool that is assigned its HDL-C value by ultracentrifugation.

The HDL-C value is traceable to the CDC reference method for determination of HDL-C. The Lipid Calibrator is manufactured targeting the HDL-C concentration at 40-80 mg/dL.

The Lipid Calibrator is stable for 5 days at +2 to +10 °C, after reconstitution.

d. *Detection limit:*
The analytical sensitivity (lower detection limit) was assessed using the zero standard (saline) and serial dilutions of in-house control serum. All the samples were assayed in 10 replicates and a mean value and SD were calculated for each sample. The analytical sensitivity (lower detection limit) was determined as HDL-C concentration of which mean -2.6 SD does not overlap with the mean +2.6 SD of the zero standard (saline). The claimed

analytical sensitivity is 0.60 mg/dL.

e. Analytical specificity:
Ascorbate, hemoglobin, conjugated bilirubin, unconjugated bilirubin, and Intralipid were added to the base serum at high concentrations. The base serum containing each substance was then serially diluted with another aliquot of base serum to which saline was added with the volume as each substance to adjust concentration of HDL-C. All dilutions were prepared to have serially diluted substance but the same concentration of HDL-C. All recoveries ranged between 97% and 101%, resulting in no interference up to 50 mg/dL ascorbate, 1000 mg/dL hemoglobin, 60 m/dL conjugated bilirubin, 60 mg/dL unconjugated bilirubin, and 1.0% Intralipid.

f. Assay cut-off:
Not applicable

2. Comparison studies:
   a. Method comparison with predicate device:
   One hundred and fifty (150) samples of non-pooled male and female human donor serum were compared by the two test systems, the HDL-EX SEIKEN Assay and the Ultra N-Geneous HDL Cholesterol Assay, both on the Hitachi 917. Each sample had an analyte concentration within the measuring range of both test devices. The samples ranged from 23.1 to 104.3 mg/dL on the subject test method. The linear regression analysis showed the following: $y = 1.04x + 0.02$, $r = 0.991$.

   Using the two diagnostic cut-points (40 and 60 mg/dL) recommended in the Adult Treatment Panel (ATP) III by the National Cholesterol Education Program, agreement between the two devices was further evaluated. At 40 mg/dL, 148 out of 150 samples were in complete agreement, resulting in accuracy by correlation of 99.2%. At 60 mg/dL, 143 out of 150 samples were in complete agreement, resulting in accuracy by correlation of 94.2%.

   b. Matrix comparison:
   Comparison studies were performed using 20 samples each of serum, heparinized plasma, and EDTA plasma. The regression results from the serum-heparinized plasma comparison were as follows: $y = 1.01x – 1.03$, $r^2 = 0.997$. The regression results from the serum-EDTA plasma comparison were as follows: $y = 1.01x – 0.85$, $r^2 = 0.996$. The results demonstrate equivalence of HDL-EX SEIKEN Assay results in all three specimen types.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable

b. Clinical specificity:
Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):
Accuracy of the HDL-EX SEIKEN Assay Kit has been verified by comparison to the reference method (RM) for HDL cholesterol (removal of chylomicrons and VLDL by ultracentrifugation, precipitation of apoB containing lipoproteins with heparin plus $MnCl_2$ and quantification of cholesterol in HDL by Abell-Kendall Cholesterol Reference Method). Forty-five (45) samples were run in duplicate on the RM and the subject device using a Hitachi 917 analyzer. The HDL-C values, as determined by the RM, ranged from 20.2 mg/dL to 63.5 mg/dL. The summary data are as follows:

Average bias: 1.1%
Among-run CV (20 days, mean 51.1 mg/dL): 0.8%
Among-run total error: 2.7%
Regression analysis: $y = 0.99x + 0.83$ mg/dL, $r^2 = 0.995$

4. Clinical cut-off:
Not applicable

5. Expected values/Reference range:

HDL cholesterol distribution in apparently healthy adults, 161 healthy men with age distribution of 19-63 years (mean 38.8 years) and 183 women with age distribution of 18-58 years (mean 37.3 years), was studied with the HDL-EX SEIKEN Assay Kit. The expected values for serum HDL-C were found as follows:

Males:	37 – 74 mg/dL
Females:	41 – 94 mg/dL

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.